Boosting p53 with interferon and viruses

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Interferon-α/β proteins are vital for innate immune responses to viruses. The tumor suppressor p53 mediates cell cycle arrest and apoptosis. A recent study in *Nature* reports that interferon-α/β stimulates p53 synthesis, demonstrating a hitherto unrecognized link.

Until recently, if someone had asked whether there was a relationship between interferons (IFNs) and p53, the answer would probably have been vague. Certainly both IFNs and p53 affect cell division, so their actions could intersect. Besides, everything in the universe is somehow interconnected, right? End of discussion. Now, a new study by Takaoka and coworkers[1] from the laboratory of Tada Taniguchi, published in the 31 July issue of *Nature*, suggests that the responses elicited by members of the IFN-α/β family and by p53 are in fact very closely connected. The study, chock-full of data and unexpected results, shows that IFN-α/β stimulates transcription of the gene encoding p53, resulting in an increase in cellular p53 protein abundance.

IFNs are cytokines that include the multigene IFN-α/β family and IFN-γ. IFN-α/β proteins are induced in the body in response to viral infections and are best known for their ability to induce synthesis of cellular proteins that mediate resistance to viruses[2,3]. In addition, IFNs affect many other cellular functions, such as cell growth, and they have immunomodulatory activities. The main function of p53 is to induce the expression of genes that cause cell cycle arrest and apoptosis, thereby preventing the proliferation of aberrant or malignant cells[4,5]. In cells, p53 is present in a dormant form, and requires activation to become fully functional. Activation of p53 is induced by agents that produce DNA damage or other forms of 'cell stress', such as ionizing radiation, chemotherapeutic drugs or aberrant cell growth signals. Takaoka *et al.*[1] show that although IFN-α/β does not produce p53 activation, the increased concentrations of p53 protein that accumulates in cells as a result of IFN-α/β stimulation can apparently bring about enhanced cellular responses to stress signals that activate p53. They then show that virus infection, too, can boost p53 accumulation, and, moreover, virus infection can result in p53 activation, which may lead to enhanced virus-induced apoptosis. Elimination of virus-infected cells limits the ability of the virus to replicate and can thus be viewed as a previously unknown defense mechanism against viruses.

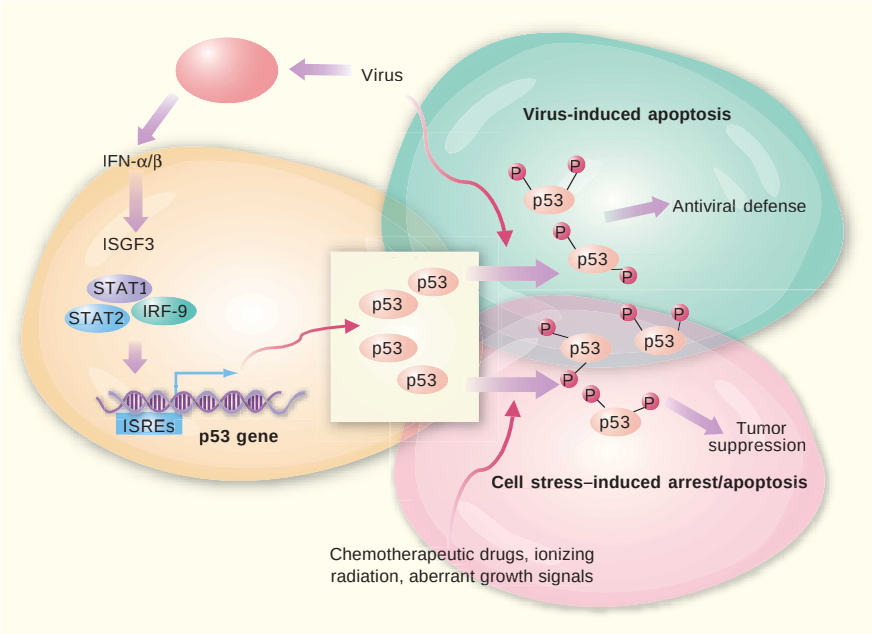

Figure 1 Stimulation of p53 gene transcription by IFN-α/β and p53 activation by virus infection. IFN-α/β proteins, produced in response to virus infection, bind to the IFN-α/β receptor on target cells, which leads to the formation of the heterotrimeric complex ISGF3, composed of activated STAT1, STAT2 and IRF-9. ISGF-3 was shown to bind to two ISRE sites in the gene encoding p53, thus activating its transcription and p53 protein synthesis[1]. Subsequent p53 activation (which involves serine phosphorylation in addition to other modifications[5]) can occur as a result of the action of various chemical signals that produce DNA damage or other forms of cell stress. Takaoka *et al.*[1] have shown that virus infection also can lead to the activation of p53, which, in turn, can produce apoptotic cell death of the virus-infected cells, resulting in the curtailing of virus replication.

At the core of the findings reported by Takaoka *et al.*[1] is the observation that IFN-β increased the level of p53 in cultured mouse cells. As it had been believed that the amount of p53 protein in cells is determined mainly by the rate at which it is degraded, rather than its rate of synthesis[5,6], the authors checked whether IFN-β affects p53 protein degradation. They found no difference between IFN-treated and untreated cells. However, they did find that expression of the gene for p53 is transcriptionally induced by IFN-β, so, unexpectedly, it is enhanced synthesis that accounts for the increase in p53 protein. Moreover, it seems that the stimulation of p53 transcription is similar to the conventional mechanism whereby IFN-α/β action produces transcriptional activation. Specifically, IFNs stimulate gene expression by activating the Jak tyrosine kinases–signal transducers and activators of transcription (Jak-STAT) pathway[7].

Binding of IFN-α or IFN-β to its heterodimeric receptor results in the activation of the receptor-associated kinases Jak1 and Tyk2, which is followed by tyrosine phosphorylation of the STAT1 and STAT2 proteins. The activated STAT proteins, together with IFN-regulatory factor 9 (IRF-9), then form the trimeric IFN-stimulated gene factor 3 (ISGF-3) complex, which translocates to the nucleus and binds to the IFN-stimulated response elements (ISREs) present in most

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genes responsive to IFN-α and IFN-β. Takaoka *et al.*[1] found that mouse and human p53 genes contain ISRE sites, and they provide evidence that transcriptional activation of p53 is likely mediated by these elements (**Fig. 1**). However, transcriptional activation of p53 is somewhat less strong than the activation of some other IFN-inducible genes. IFN-γ, a 'cousin' of IFN-α/β that uses the Jak-STAT signaling pathway but generally does not activate genes through the ISRE, failed to activate p53 transcription.

Takaoka *et al.*[1] also provide evidence that the boosting of p53 amounts by IFN is functionally relevant. Although IFN-β treatment alone did not produce p53 activation, the enhanced accumulation of p53 in cells treated with IFN-β rendered them more responsive to apoptosis induced by X-irradiation[1]. Other evidence of biologic relevance includes the demonstration that the apoptotic response to the chemotherapeutic agent 5-fluorouracil (5-FU) is enhanced in IFN-β-treated cells and that IFN-β reduced, in a p53-dependent way, the growth of transformed colonies of mouse fibroblasts expressing the oncogenes E6 and Ha-Ras.

It has been generally known that products of some oncogenic viruses (for example, papillomavirus E6 protein or simian virus 40 large T antigen) bind to and inactivate p53 in the cell, in some cases causing p53 degradation, and that this action accounts for the transforming capacity of such viruses[4,5]. Hence, at least for oncogenic viruses, their relationship with p53 has generally been viewed as antagonistic. The most unexpected and intriguing aspect of the work by Takaoka and colleagues[1] probably is that they find evidence for a stimulation of p53 expression in cells infected with three types of conventional viruses: vesicular stomatitis virus, Newcastle disease virus and herpes simplex virus. The boosting of p53 protein by at least one of these viruses (vesicular stomatitis virus) was apparently secondary to virus-induced IFN production, as it was not found in cells that are unresponsive to IFN-β. However, virus infection also produced p53 activation, which was not found in cells treated with IFN in the absence of virus infection (**Fig. 1**).

Evidence for p53 activation by virus infection was based on increased serine phosphorylation, an increased expression of some genes that are known to be transcriptionally activated by p53 (*Mdm2* and *Puma*) and, perhaps most convincingly, by virus-induced apoptosis that was notable in wild-type mouse fibroblasts but much less prominent in p53-deficient fibroblasts. The authors propose that activation of p53 and induction of apoptosis by virus infection represents a previously unknown antiviral defense mechanism, as 'altruistic suicide' of virus-infected cells would reduce the yield of progeny virus. In support of this idea, they show that p53-null mice had a much higher death rate after vesicular stomatitis virus infection than did wild-type mice (100% versus less than 20% mortality). Virus titers in the serum were also much higher in p53-deficient mice than in normal mice.

The work of Takaoka *et al.*[1] demonstrates new links between antiviral host defenses and tumor suppression. IFNs are known to inhibit the growth of many cells and, as is well known, IFNs do show antitumor activity in experimental animals and sometimes also in humans[8,9]. Many of the *in vivo* actions are likely to be the result of immunomodulation by IFNs but, as pointed out by Takaoka *et al.*[1], some of the antitumor actions may involve p53 induction by IFN. Another thought concerns the possible implication of these findings for the combined therapeutic use of IFN and chemotherapeutic drugs that can activate p53, such as 5-FU. Of course, it remains to be seen whether the IFN-mediated enhancement of the apoptotic response to 5-FU, seen in cells in culture, can be reproduced in the intact organism. The provocative work of Takaoka *et al.*[1] will undoubtedly inspire follow-up studies that will further explore the proposed links between IFN and p53 and their relevance to antiviral and antitumor defenses.

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